8EHQ-0702-15175

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MR 60815

July 26, 2002

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Attn: TSCA 8(e) Coordinator (Room G99 East Tower)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460-0001

Re: Subsituted Carbomonocyclic Ether

To Whom It May Concern:

This letter will serve as a TSCA 8(e) Notification for a potential increased risk to human health regarding subsituted carbomonocyclic ether (Confidential Business Information attached).

A mouse micronucleus study was conducted on the aforementioned material. The results showed genotoxic potential for this chemistry. A final report is attached.

This chemistry is an industrial material. Physicochemical properties and engineering controls for the purpose of minimizing human exposure are under review. Appropriate warnings are in place to comply with OHSA Hazard Communication Standards 29 CFR 1910.1200.

If you have any questions please contact me.

Sincerely,

Janet C. Gould, Ph.D., D.A.B.T.
Senior Toxicologist
Product Assurance and Regulatory Affairs

COMPANY SANITIZED

JCG/jlm
Attachments
cc: P. Mudge

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CONFIDENTIAL BUSINESS INFORMATION

MR 604315

SafePharm Laboratories

CONFIDENTIAL [ ]

MICRONUCLEUS TEST IN THE MOUSE

SPL PROJECT NUMBER: 1614/001

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STUDY SPONSOR:

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DE1 2BT
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QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	26 July 2000	Standard Test Method Compliance Audit
	27 February 2002	Test Material Preparation
	27 February 2002	Animal Preparation
	27 February 2002	Dosing
	27 February 2002	Assessment of Response
	01, 22 February 2002	Cell Harvest/Staining/Slide Preparation
§	04 April 2002	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

G. Wren ..... DATE: 16 MAY 2002 .....

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**

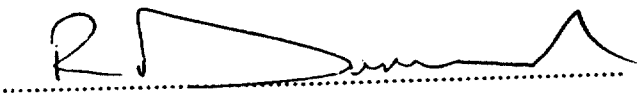
Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA; R Hurst MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.



DATE: 15 MAY 2002

R Durward HNC
Study Director

CONTENTS

CONFIDENTIAL [ ]

MICRONUCLEUS TEST IN THE MOUSE

SUMMARY

Introduction. The study was performed to assess the potential of the test material to produce damage to chromosomes or aneuploidy when administered to mice. The method was designed to comply with the UKEMS Sub-committee on Guidelines for Mutagenicity Testing, Report, Part 1 revised (Basic Mutagenicity Tests: UKEMS recommended procedures, 1990). The study design also complies with the revised OECD Guidelines for Testing of Chemicals No.474 "Micronucleus Test", Method B12 of the EEC Commission Directive 2000/32/EEC, the USA EPA, TSCA and FIFRA guidelines and the Japanese METI/MHLW guidelines for testing of new chemical substances.

Methods. A range-finding study was performed to find suitable dose levels of the test material, route of administration and investigate to see if there was a marked difference in toxic response between the sexes. There was no marked difference in test material toxicity between the sexes, therefore the main study was performed using only male mice. The micronucleus study was conducted using the intraperitoneal route in groups of seven mice (males) at the maximum tolerated dose (MTD) of 1000 mg/kg with 500 and 250 mg/kg as the two lower dose levels. Animals were killed 24 or 48 hours later, the bone marrow extracted and smear preparations made and stained. Polychromatic (PCE) and normochromatic (NCE) erythrocytes were scored for the presence of micronuclei.

Further groups of mice were given a single intraperitoneal dose of arachis oil (7 mice) or dosed orally with cyclophosphamide (5 mice), to serve as vehicle and positive controls respectively. Vehicle control animals were killed 24 or 48 hours later, and positive control animals were killed after 24 hours.

Results. Decreases in the PCE/NCE ratio were observed in the 24 and 48-hour 1000 mg/kg test material dose groups when compared to their concurrent control groups and a decrease was also observed at 250 mg/kg, but not at 500 mg/kg, in the 24-hour test material group. None of the decreases in PCE/NCE ratio achieved statistical significance. However, the decreases in PCE/NCE ratio and the presence of clinical signs were taken to indicate that systemic absorption had occurred.

There was evidence of statistically significant increases in the incidence of micronucleated polychromatic erythrocytes in animals dosed with the test material when compared to the concurrent vehicle control group in the 24-hour exposure groups. In the 48-hour 1000 mg/kg test material dose group, there was a marked increase in the incidence of micronucleated polychromatic erythrocytes. However, due to the large standard deviation value the increase was not statistically significant. Animals 21 and 24 showed extraordinarily high values for micronucleated polychromatic erythrocytes and also elevated values for micronucleated normochromatic erythrocytes. It was considered that the increases in the frequency of micronucleated cells was toxicologically significant. There was a high degree of inter-animal variation, both in terms of PCE/NCE ratio and the incidence of micronucleated cells. It was considered that this may have been the result of inter-animal differences in absorption of the test material.

The positive control material produced a marked increase in the frequency of micronucleated polychromatic erythrocytes.

Conclusion. The test material was considered to be genotoxic under the conditions of the test.

CONFIDENTIAL [ ]

MICRONUCLEUS TEST IN THE MOUSE

1. INTRODUCTION

The micronucleus test is a mammalian *in vivo* test which detects damage to the chromosomes induced by chemicals. In addition, numerical changes due to chromosome loss during cell division can be detected in this test.

The study was performed according to a method which followed the UKEMS Sub-committee on Guidelines for Mutagenicity Testing, Report, Part 1 revised (Basic Mutagenicity Tests: UKEMS recommended procedures, 1990). The study design also complies with the revised OECD Guidelines for Testing of Chemicals No.474 "Micronucleus Test", Method B12 of the EEC Commission Directive 2000/32/EEC, the USA EPA, TSCA and FIFRA guidelines and the Japanese METI/MHLW guidelines for testing of new chemical substances. The results of the test are believed to be of value in predicting the mutagenic potential of the test material to man. The test system was chosen because the mouse has been shown to be a suitable model for this type of study and is recommended in the test method.

The experimental phases of the study were performed between 16 January 2002 and 19 February 2002.

2. PRINCIPLES OF INVESTIGATION

In mitotic cells in which chromosome damage has been caused by the test material or its metabolites, fragments (centric or acentric) or whole chromosomes tend to lag behind in the anaphase stage of cell division. After telophase, a large proportion of the fragments are not included in the nuclei of the daughter cells and hence form a single or multiple micronuclei (Howell-Jolly bodies) in the cytoplasm of these cells. These micronuclei are seen in a wide variety of cell types, but erythrocytes are chosen since micronuclei are easily detected in these cells.

A few hours after the last mitosis is completed, erythrocytes expel their nuclei. Immature erythrocytes, less than 24 hours old, stain blue with May-Grünwald/Giemsa due to the presence of minute fragments of nuclear material in the cytoplasm. This material is mainly ribonucleic acid (RNA), which gradually disappears so that more mature erythrocytes (normochromatic erythrocytes) stain pink with May-Grünwald/Giemsa. The immature blue staining cells are

known as polychromatic erythrocytes and mauve stained micronuclei are easily detected in this cell type. If scoring is restricted to polychromatic erythrocytes, all the chromosomal damage detected will have been caused during the final cell cycle of the nucleated precursor cells. Thus by examining polychromatic cells at various periods after administration, the effect of the test material over the previous 30 hours can be monitored.

Any toxic effects of the test material on the immature nucleated cells may lead to a reduction in cell division and cell death. This in turn leads to a reduction in cell volume and to compensate for this, peripheral blood is shunted into the bone marrow. If the ratio of polychromatic to normochromatic erythrocytes is scored and found to be significantly lower than the control value, this is taken as being indicative of cytotoxicity.

3. TEST AND CONTROL MATERIALS AND EXPERIMENTAL PREPARATION

3.1 Test Material

Sponsor's identification	:	[ ] CONFIDENTIAL
Description	:	yellow slightly viscous liquid
Batch number	:	1000000768
Date received	:	14 December 2001
Storage conditions	:	-18°C in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

For the purpose of this study the test material was freshly prepared as required as a solution at the appropriate concentration in arachis oil.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Standard Test Method.

3.2 Positive Control Material

The positive control material was supplied by Sigma Chemicals, as follows:

Supplier's identification	:	Cyclophosphamide
Supplier's lot number	:	91K1176
Safepharm serial number	:	R-2326
Date received	:	30 October 2001
Storage conditions	:	4°C in the dark

For the purpose of this study the positive control material was freshly prepared as required as a solution at the appropriate concentration in distilled water (Norton Healthcare batch no. A1199).

The concentration, homogeneity and stability of the positive control material and its preparation were not determined by analysis.

3.3 Vehicle Control

The vehicle was supplied by Analytical Supplies Ltd, as follows:

Supplier's identification	:	Arachis oil BP
Supplier's lot number	:	T53
Safepharm serial number	:	V-2140
Date received	:	20 March 2001
Description	:	straw coloured viscous liquid
Storage conditions	:	room temperature

4. METHODS

4.1 Animals and Animal Husbandry

Sufficient male albino Crl:CD-1™(ICR)BR strain mice were supplied by Charles River (UK) Limited, Margate, Kent. At the start of the main study the mice weighed 24 to 29g and were approximately five to eight weeks old. After a minimum acclimatisation period of seven days the animals were selected at random and given a number unique within the study by ear punching and a number written on a colour coded cage card.

The animals were housed in groups of up to seven in solid-floor polypropylene cages with woodflake bedding. Free access to mains drinking water and food (Certified Rat and Mouse Diet 5LF2, PMI Nutrition International, Nottingham, UK) was allowed throughout the study.

The temperature and relative humidity were set to achieve limits of 19 to 25°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was approximately fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours light and twelve hours darkness.

4.2 Procedure

4.2.1 Range-finding Toxicity Study

A range-finding toxicity study was performed to determine a suitable dose level and route of administration for the micronucleus study. The dose level selected should ideally be the maximum tolerated dose level or that which produces some evidence of cytotoxicity up to a maximum recommended dose of 2000 mg/kg. The range-finding toxicity study was also used to determine if the main study was to be performed using both sexes or males only.

Groups of mice were dosed as follows:

Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Mice	
			Male	Female
2000 oral	200	10	2	2
2000 ip	200	10	2	2
1000 ip	100	10	2	2

All animals were dosed once only at the appropriate dose level by gavage using a metal cannula or with a hypodermic needle attached to a graduated syringe. The volume administered to each animal was calculated according to its bodyweight at the time of dosing.

Animals were observed one hour after dosing and subsequently once daily for two days. Any deaths and evidence of overt toxicity were recorded at each observation. No necropsies were performed.

ip = Intraperitoneal

4.2.2 Micronucleus Study

Groups, each of seven mice, were dosed once only via the intraperitoneal route with the test material at 1000, 500 or 250 mg/kg. One group of mice from each dose level was killed by cervical dislocation 24 hours following treatment and a second group dosed with 1000 mg/kg was killed after 48 hours. In addition, three further groups of mice were included in the study; two groups (seven mice) were dosed via the intraperitoneal route with the vehicle alone (arachis oil) and a third group (five mice) was dosed orally with cyclophosphamide, a positive control material known to produce micronuclei under the conditions of the test. The vehicle controls were killed 24 or 48 hours following dosing and positive control group animals were killed 24 hours following dosing. The experimental design is summarised as follows:

Dose Group	Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Kill Time (Hours After Dosing)	Animal Numbers
1. Vehicle Control (Arachis oil)	0	0	10	48	1 - 7
2. Vehicle Control (Arachis oil)	0	0	10	24	8 -14
3. Positive Control (Cyclophosphamide)	50	5	10	24	15 - 19
4. [redacted]	1000	100	10	48	20 - 26
5. [redacted]	1000	100	10	24	27 - 33
6. [redacted]	500	50	10	24	34 - 40
7. [redacted]	250	25	10	24	41 - 47

All animals were observed for signs of overt toxicity and death one hour after dosing and then once daily as applicable and immediately prior to termination.

4.2.3 Slide Preparation

Immediately following termination (*ie.* 24 or 48 hours following dosing), both femurs were dissected from each animal, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed in absolute methanol and stained in May-Grünwald/Giemsa, allowed to air-dry and coverslipped using mounting medium.

4.2.4 Slide Evaluation

Stained bone marrow smears were coded and examined blind using light microscopy at x1000 magnification. The incidence of micronucleated cells per 2000 polychromatic erythrocytes (PCE-blue stained immature cells) per animal was scored. Micronuclei are normally circular in shape, although occasionally they may be oval or half-moon shaped, and have a sharp contour with even staining. In addition, the number of normochromatic erythrocytes (NCE-pink stained mature cells) associated with 1000 erythrocytes were counted; these cells were also scored for incidence of micronuclei.

The ratio of polychromatic to normochromatic erythrocytes was calculated together with appropriate group mean values and standard deviations.

4.2.5 Interpretation of Results

A comparison was made between the number of micronucleated polychromatic erythrocytes occurring in each of the test material groups and the number occurring in the corresponding vehicle control group.

A positive mutagenic response was demonstrated when a statistically significant, dose-responsive, toxicologically relevant increase in the number of micronucleated polychromatic erythrocytes was observed for either the 24 or 48-hour kill times when compared to their corresponding control group.

If these criteria were not demonstrated, then the test material was considered to be non-genotoxic under the conditions of the test.

A positive response for bone marrow toxicity was demonstrated when the dose group mean polychromatic to normochromatic ratio was shown to be statistically significantly lower than the concurrent vehicle control group.

All data were statistically analysed using appropriate statistical methods as recommended by the UKEMS Sub-committee on Guidelines for Mutagenicity Testing Report, Part III (1989). The data was analysed following a $\sqrt{(x+1)}$ transformation using Student's t-test (two tailed) and any significant results were confirmed using the one way analysis of variance.

5. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

6. RESULTS

6.1 Range-finding Toxicity Study

The mortality data are summarised as follows:

Dose Level (mg/kg)	Sex	Number of Animals Treated	Route	Deaths on Day			Total Deaths
				0	1	2	
2000	Male	2	oral	0	0	0	0/4
	Female	2		0	0	0	
2000	Male	2	ip	0	2	0	4/4
	Female	2		1	1	0	
1000	Male	2	ip	0	0	0	0/4
	Female	2		0	0	0	

In animals dosed with test material via the intraperitoneal route premature deaths occurred at 2000 mg/kg. At 1000 mg/kg no premature deaths occurred and clinical signs were observed as follows: hunched posture, lethargy, body tremors, splayed gait, ptosis and pilo-erection.

In animals dosed with the test material via the oral route no premature deaths occurred and clinical signs were observed as follows: hunched posture and lethargy.

The test material showed no marked difference in its toxicity to male or female mice, it was therefore considered to be acceptable to use males only for the main study. Adequate evidence of test material toxicity was only demonstrated via the intraperitoneal route of administration, therefore, this was selected for use in the main study. The maximum tolerated dose (MTD) of the test material, 1000 mg/kg, was selected for use in the main study, with 500 and 250 mg/kg as the lower dose levels.

6.2 Micronucleus Study

6.2.1 Mortality Data and Clinical Observations

There were no premature deaths seen in any of the dose groups. Clinical signs were observed in animals dosed with the test material at 1000 mg/kg in both the 24 and 48-hour groups where applicable, these were as follows: lethargy, splayed gait, hunched posture, and ptosis.

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ip = Intraperitoneal

6.2.2 Evaluation of Bone Marrow Slides

A summary of the results of the micronucleus study is given in Table 1. Individual and group mean data are presented in Tables 2 to 8.

Decreases in the PCE/NCE ratio were observed in the 24 and 48-hour 1000 mg/kg test material dose groups when compared to their concurrent control groups and a decrease was also observed at 250 mg/kg, but not at 500 mg/kg, in the 24-hour test material group. None of the decreases in PCE/NCE ratio achieved statistical significance. However, the decreases in PCE/NCE ratio and the observation of clinical signs were taken to indicate that systemic absorption had occurred.

There was evidence of statistically significant increases in the incidence of micronucleated polychromatic erythrocytes in animals dosed with the test material when compared to the concurrent vehicle control group in the 24-hour exposure groups. In the 48-hour 1000 mg/kg test material dose group, there was a marked increase in the incidence of micronucleated polychromatic erythrocytes. However, due to the large standard deviation value the increase was not statistically significant. Animals 21 and 24 showed extraordinarily high values for micronucleated polychromatic erythrocytes and also elevated values for micronucleated normochromatic erythrocytes. It was considered that the increases in the frequency of micronucleated cells was toxicologically significant. There was a high degree of inter-animal variation, both in terms of PCE/NCE ratio and the incidence of micronucleated cells. It was considered that this may have been the result of inter-animal differences in absorption of the test material.

The positive control group showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test.

The test material was found to produce a significant increase in the frequency of micronuclei in polychromatic erythrocytes of mice under the conditions of the test.

7. CONCLUSION

The test material was considered to be genotoxic under the conditions of the test.

CONFIDENTIAL [ ] MICRONUCLEUS TEST IN THE MOUSE

Table 1 Micronucleus Study - Summary of Group Mean Data

TREATMENT GROUP	NUMBER OF PCE WITH MICRONUCLEI PER 2000 PCE		PCE/NCE RATIO	
	GROUP MEAN	SD	GROUP MEAN	SD
1. Vehicle Control 48-hour sampling time	2.4	2.1	0.84	0.24
2. Vehicle Control 24-hour sampling time	1.1	1.6	0.84	0.21
3. Positive Control 24-hour sampling time	28.4***	13.2	1.20	0.24
4. [ ] 1000 mg/kg 48-hour sampling time	28.0	50.8	0.64	0.40
5. [ ] 1000 mg/kg 24-hour sampling time	9.0***	5.0	0.66	0.19
6. [ ] 500 mg/kg 24-hour sampling time	2.6*	0.8	0.90	0.30
7. [ ] 250 mg/kg 24-hour sampling time	9.0	16.8	0.67	0.27

PCE = Polychromatic erythrocytes
NCE = Normochromatic erythrocytes
SD = Standard deviation
* = $P < 0.05$
*** = $P < 0.001$

Table 2 Micronucleus Study - Individual and Group Means and Standard Deviations: Vehicle Control (10 ml/kg) 48-Hour Sampling Time

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		PCE/NCE RATIO
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
1. VEHICLE CONTROL 10 ml/kg 48-hour sampling time	1	28	2000	1	0.05	556	0	0.80
	2	27	2000	0	0.00	601	0	0.66
	3	27	2000	4	0.20	558	2	0.79
	4	26	2000	4	0.20	578	0	0.73
	5	28	2000	0	0.00	422	0	1.37
	6	28	2000	3	0.15	552	0	0.81
	7	28	2000	5	0.25	573	1	0.75
	Group Mean	27.4	2000	2.4	0.12	549	0.4	0.84
	SD	0.8	0	2.1	0.10	58	0.8	0.24

Table 3 **Micronucleus Study - Individual and Group Means and Standard Deviations: Vehicle Control (10 ml/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		PCE/NCE RATIO
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
2. VEHICLE CONTROL 10 ml/kg 24-hour sampling time	8	28	2000	0	0.00	585	0	0.71
	9	27	2000	4	0.20	663	0	0.51
	10	28	2000	0	0.00	494	0	1.02
	11	29	2000	0	0.00	533	1	0.88
	12	27	2000	2	0.10	475	0	1.11
	13	28	2000	0	0.00	590	0	0.69
	14	28	2000	2	0.10	519	0	0.93
	Group Mean	27.9	2000	1.1	0.06	551	0.1	0.84
	SD	0.7	0	1.6	0.08	65	0.4	0.21

Table 4 **Micronucleus Study - Individual and Group Means and Standard Deviations: Cyclophosphamide (50 mg/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		PCE/NCE RATIO
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
3. CYCLOPHOSPHAMIDE 50 mg/kg 24-hour sampling time	15	27	2000	22	1.10	536	2	0.87
	16	28	2000	18	0.90	427	1	1.34
	17	24	2000	19	0.95	479	0	1.09
	18	25	2000	49	2.45	402	0	1.49
	19	26	2000	34	1.70	454	1	1.20
	Group Mean	26.0	2000	28.4	1.42	460	0.8	1.20
	SD	1.6	0	13.2	0.66	52	0.8	0.24

Table 5 **Micronucleus Study - Individual and Group Means and Standard Deviations: Test Material (1000 mg/kg) 48-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		PCE/NCE RATIO
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
[4.] 1000 mg/kg 48-hour sampling time	20	27	2000	1	0.05	519	0	0.93
	21	28	2000	138	6.90	778	17	0.29
	22	28	2000	7	0.35	636	3	0.57
	23	26	2000	3	0.15	664	0	0.51
	24	28	2000	43	2.15	917	8	0.09
	25	27	2000	2	0.10	542	0	0.85
	26	27	2000	2	0.10	444	0	1.25
	Group Mean	27.3	2000	28.0	1.40	643	4.0	0.64
	SD	0.8	0	50.8	2.54	163	6.5	0.40

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Table 6 **Micronucleus Study - Individual and Group Means and Standard Deviations: Test Material (1000 mg/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		PCE/NCE RATIO
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
[5.] 1000 mg/kg 24-hour sampling time	27	28	2000	8	0.40	720	1	0.39
	28	28	2000	6	0.30	575	2	0.74
	29	28	2000	12	0.60	514	0	0.95
	30	27	2000	19	0.95	652	0	0.53
	31	27	2000	6	0.30	610	0	0.64
	32	28	2000	5	0.25	653	2	0.53
	33	28	2000	7	0.35	548	0	0.82
	Group Mean	27.7	2000	9.0	0.45	610	0.7	0.66
	SD	0.5	0	5.0	0.25	71	1.0	0.19

Table 7 **Micronucleus Study - Individual and Group Means and Standard Deviations: Test Material (500 mg/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		PCE/NCE RATIO
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
[6.] [ ] 500 mg/kg 24-hour sampling time	34	26	2000	3	0.15	499	0	1.00
	35	26	2000	2	0.10	498	1	1.01
	36	28	2000	2	0.10	490	0	1.04
	37	27	2000	4	0.20	724	0	0.38
	38	29	2000	2	0.10	425	0	1.35
	39	26	2000	2	0.10	570	2	0.75
	40	27	2000	3	0.15	566	0	0.77
	Group Mean	27.0	2000	2.6	0.13	539	0.4	0.90
	SD	1.2	0	0.8	0.04	95	0.8	0.30

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Table 8 **Micronucleus Study - Individual and Group Means and Standard Deviations: Test Material (250 mg/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		PCE/NCE RATIO
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
7. [ ] 250 mg/kg 24-hour sampling time	41	27	2000	2	0.10	478	0	1.09
	42	26	2000	12	0.60	706	0	0.42
	43	25	2000	1	0.05	556	1	0.80
	44	27	2000	0	0.00	672	0	0.49
	45	28	2000	0	0.00	755	0	0.32
	46	25	2000	2	0.10	558	1	0.79
	47	29	2000	46	2.30	564	1	0.77
	Group Mean	26.7	2000	9.0	0.45	613	0.4	0.67
	SD	1.5	0	16.8	0.84	99	0.5	0.27

CONFIDENTIAL [ ]MICRONUCLEUS TEST IN THE MOUSE

Appendix 1 Historical Vehicle Control Data from 60 Studies (120 Groups)

Table 1: Relative Group Frequency of Mean Micronuclei Per 1000 PCEs

Frequency of Micronuclei per 1000 PCEs	Number of Study Groups Per MN/PCE Frequency		
	24-Hour Groups	48-Hour Groups	Combined
0.0	3	3	6
0.1	1	1	2
0.2	2	6	8
0.3	0	1	1
0.4	9	10	19
0.5	0	0	0
0.6	13	10	23
0.7	2	3	5
0.8	7	5	12
0.9	3	0	3
1.0	5	2	7
1.1	2	4	6
1.2	4	6	10
1.3	2	1	3
1.4	1	1	2
1.5	0	0	0
1.6	2	6	8
1.7	0	0	0
1.8	0	0	0
1.9	0	1	1
2.0	1	0	1
2.1	0	0	0
2.2	1	0	1
2.3	1	0	1
2.4	1	0	1
Total	60	60	120

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Appendix 1 Historical Vehicle Control Data from 60 Studies (120 Groups) (continued)

Table 2: Relative Group Frequency Categories of Micronuclei Per 1000 PCEs

48-Hour Control Group (60 Groups)

Frequency Categories	Groups	
0.0 - 0.4	21	(35%)
0.5 - 0.9	18	(30%)
1.0 - 1.4	14	(23%)
1.5 - 2.0	7	(12%)
2.1 - 2.5	0	(0%)

24-Hour Control Group (60 Groups)

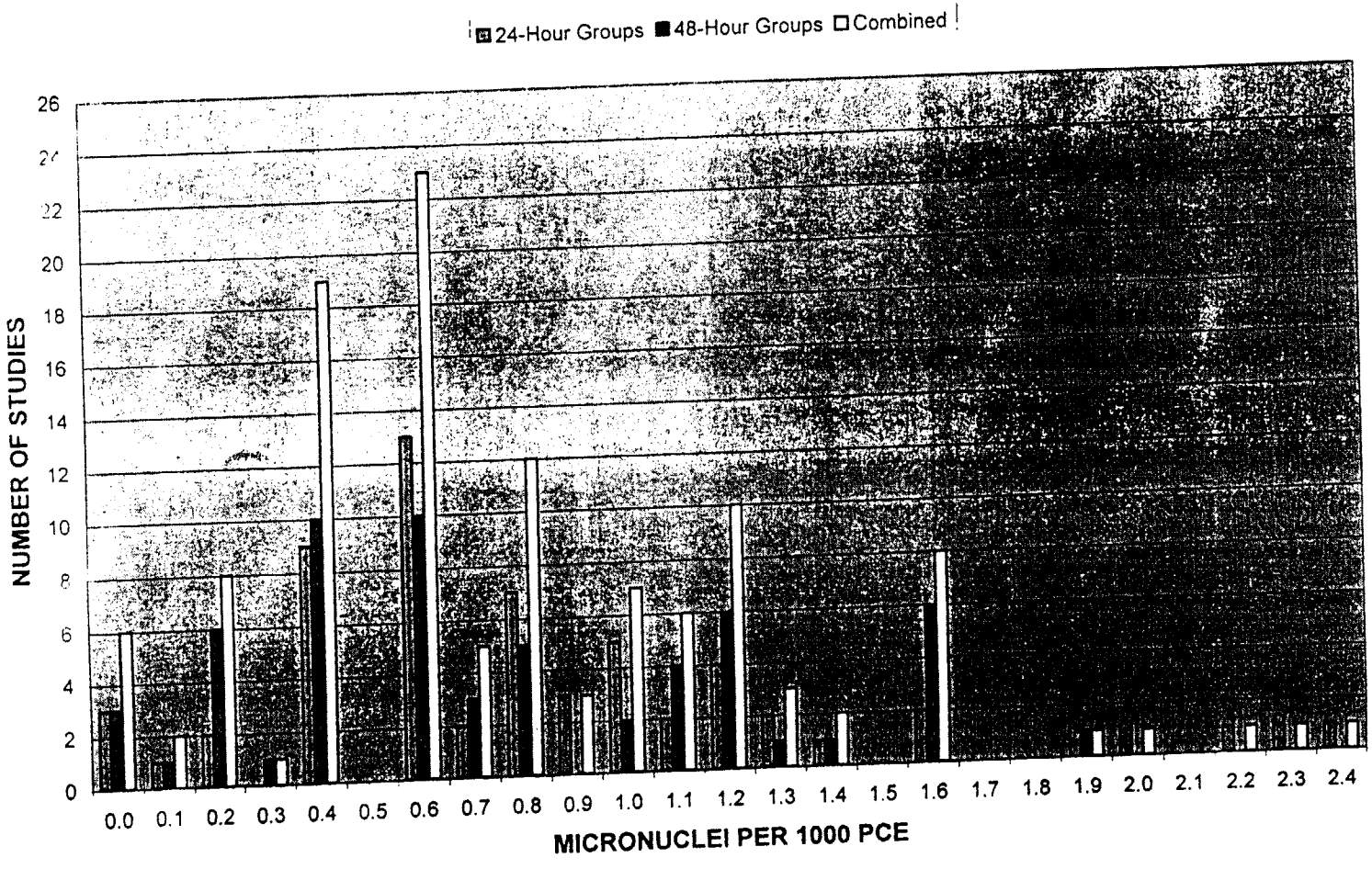
Frequency Categories	Groups	
0.0 - 0.4	15	(25%)
0.5 - 0.9	25	(42%)
1.0 - 1.4	14	(23%)
1.5 - 2.0	3	(5%)
2.1 - 2.5	3	(5%)

Combined 24 and 48-Hour Groups (120 Groups)

Frequency Categories	Groups	
0.0 - 0.4	36	(30%)
0.5 - 0.9	43	(36%)
1.0 - 1.4	28	(23%)
1.5 - 2.0	10	(8%)
2.1 - 2.5	3	(3%)

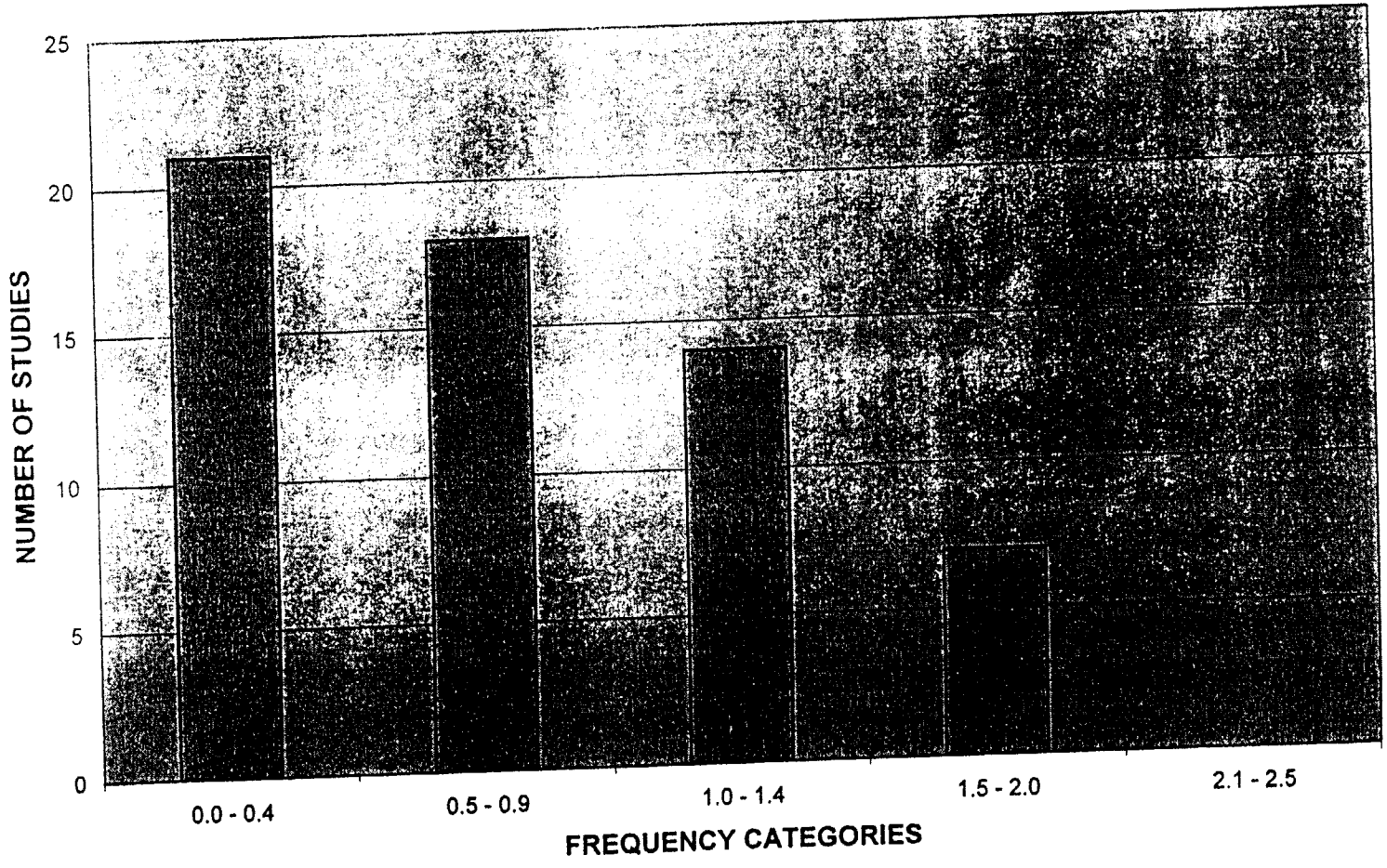
Appendix 1 **Historical Vehicle Control Data from 60 Studies (120 Groups) (continued)**

Figure 1: **Relative Group Frequency of Mean Micronuclei Per 1000 PCEs**



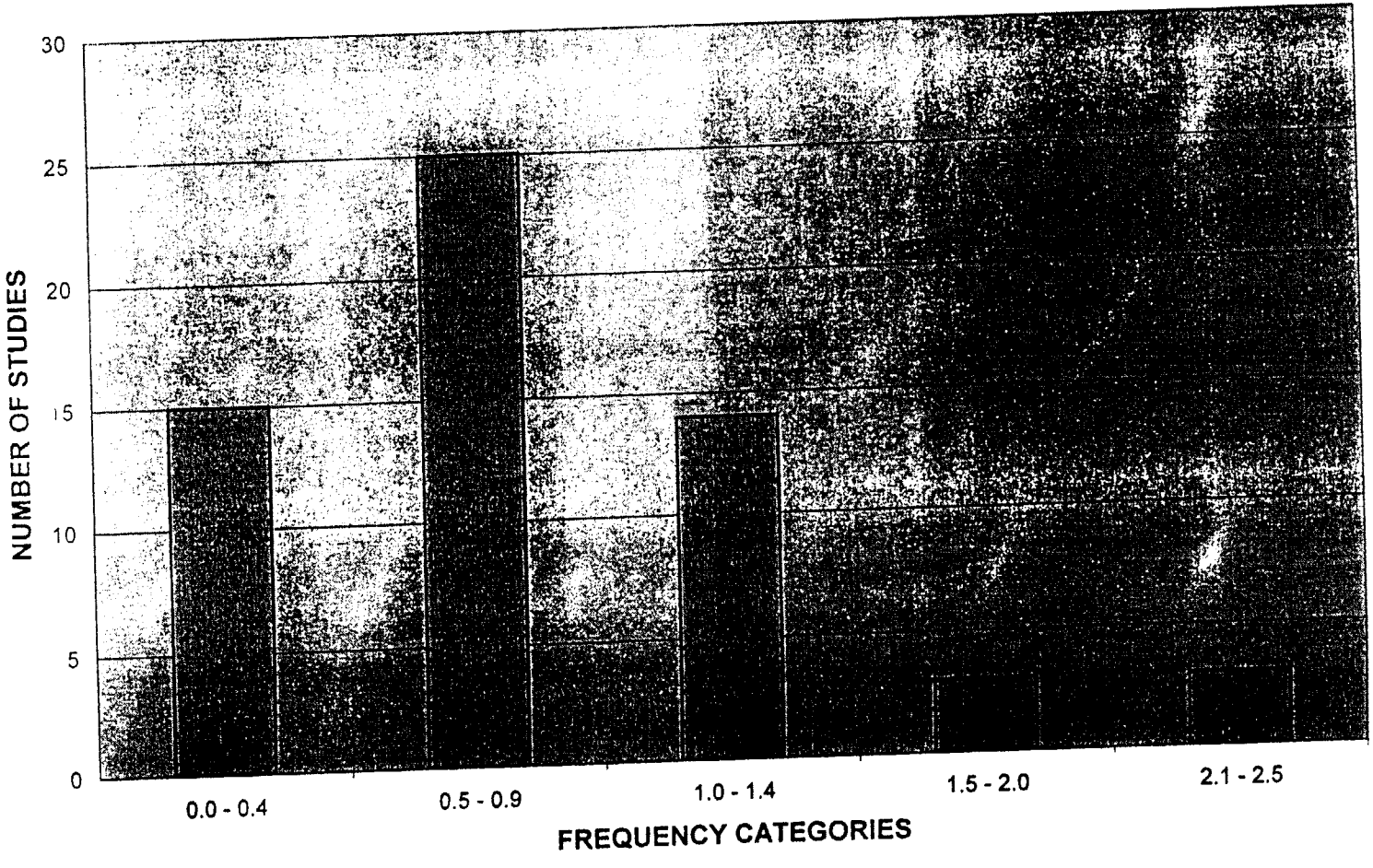
Appendix 1 **Historical Vehicle Control Data from 60 Studies (120 Groups) (continued)**

Figure 2: **Relative Group Frequency of Mean Micronuclei Per 1000 PCEs - 48-Hour Control Group**



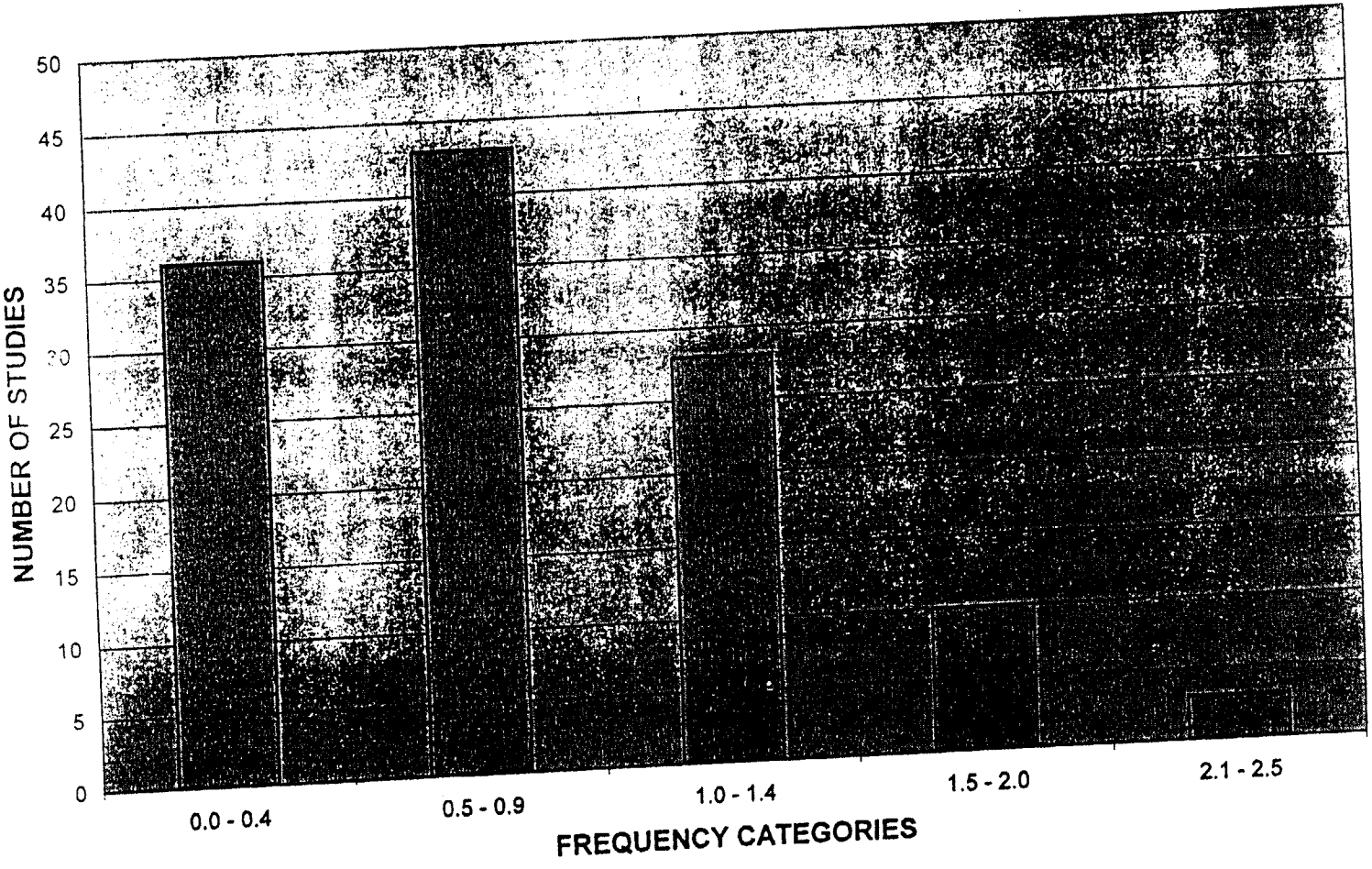
Appendix 1 **Historical Vehicle Control Data from 60 Studies (120 Groups) (continued)**

Figure 3: **Relative Group Frequency of Mean Micronuclei Per 1000 PCEs - 24-Hour Control Group**



Appendix 1 **Historical Vehicle Control Data from 60 Studies (120 Groups) (continued)**

Figure 4: **Relative Group Frequency of Mean Micronuclei Per 1000 PCEs - All Control Groups**



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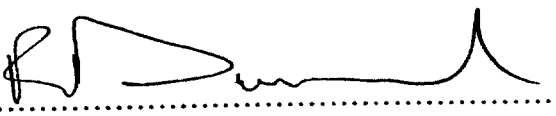
MICRONUCLEUS TEST IN THE MOUSE

SPL PROJECT NUMBER: 1614/001

I verify that this is an exact copy of the original report which is located in the Archives of SafePharm Laboratories Limited, Derby, UK.

R Durward HNC
Study Director ..... 

DATE ..... 17 MAY 2002

Appendix 2 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

SafePharm Laboratories Ltd
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD

TEST TYPE

Analytical Chemistry
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Tests
Toxicology

DATE OF INSPECTION
28 February 2000

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander 26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority